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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/876,187	06/05/2001	Stuart A. Lipton	P-LJ 4714	5845

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EXAMINER	
FALK, ANNE MARIE	
ART UNIT	PAPER NUMBER
1632	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	01/19/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	09/876,187	LIPTON ET AL.	
	Examiner	Art Unit	
	Anne-Marie Falk, Ph.D.	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 October 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-20 and 58-79* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-20 and 58-79* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *18 March 2002* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

The response filed October 4, 2006 has been entered. The amendment to the specification has been entered. The response filed February 23, 2006 (referred to herein as "the response") has been entered. Claims 59-79 have been newly added.

Claims 1-20 and 58-79 are pending in the instant application.

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114 was filed in this application after appeal to the Board of Patent Appeals and Interferences, but prior to a decision on the appeal. Since this application is eligible for continued examination under 37 CFR 1.114 and the fee set forth in 37 CFR 1.17(e) has been timely paid, the appeal has been withdrawn pursuant to 37 CFR 1.114 and prosecution in this application has been reopened pursuant to 37 CFR 1.114. Applicant's submission filed on February 23, 2006 has been entered.

Priority

Applicant's claim for domestic priority under 35 U.S.C. 119(e) is acknowledged. However, the provisional application upon which priority is claimed fails to provide adequate support under 35 U.S.C. 112 for Claims 1-20 and 58-79 of this application, for the same reasons discussed hereinbelow as applied to the present application. Application serial no. 60/209,539 fails to provide an enabling disclosure for the invention now being claimed in Claims 1-20 and 58-79, for the reasons discussed herein below as a rejection under 35 U.S.C. 112, first paragraph, as applied to the instant application.

Thus, the earlier-filed application does not meet the requirements under 35 U.S.C. 119(e) for the benefit of obtaining priority to an earlier-filed application, as the earlier-filed application does not enable the full scope of the presently claimed invention.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-20 and 58 stand rejected and Claims 59-79 are rejected under 35 U.S.C. 112, first paragraph, for reasons of record advanced on pages 2-6 of the Office Action mailed 6/16/04, on pages 2-12 of the Office Action mailed 11/29/04, and on pages 2-12 of the Office Action mailed 8/12/05, and for further reasons as discussed herein, because the specification, while being enabling for

A method of differentiating progenitor cells *in vitro*, comprising the steps of (a) contacting *in vitro* said progenitor cells with retinoic acid; and (b) introducing into said progenitor cells a nucleic acid molecule encoding an MEF2C polypeptide, wherein said progenitor cell is selected from the group consisting of a P19 cell and a mouse embryonic stem cell, thereby differentiating said progenitor cells to produce a cell population containing neuronal cells protected from apoptotic cell death,

does not reasonably provide enablement for the full scope of the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The factors to be considered in determining whether a disclosure meets the enablement requirement of 35 U.S.C. 112, first paragraph, are set forth in *In re Wands*, 8 USPQ2d 1400, at 1404 (CAFC 1988). These factors include: (1) the nature of the invention, (2) the state of the prior art, (3) the relative level of skill of those in the art, (4) the predictability of the art, (5) the breadth of the

claims, (6) the amount of direction or guidance presented, (7) the presence or absence of working examples, and (8) the quantity of experimentation necessary (MPEP 2164.01(a)).

The following factors have been considered.

Nature of the invention and scope of the claims. The claims are directed to a method of differentiating progenitor cells, particularly embryonic stem cells and hematopoietic stem cells. The claims encompass *in vivo* and *in vitro* applications of the method. The claims cover a wide variety of different types of stem cells and progenitor cells that could be used as the starting material. The progenitor cell may be in culture or may be an endogenous cell residing *in vivo*. The specification asserts that the cell compositions developed from the claimed method are useful in therapeutic transplantation. Thus, the sole asserted utility for the claimed invention is to produce a therapeutic effect. The claims are broad in scope and cover the use of any differentiating agent in combination with any progenitor cell, as well as a wide variety of MEF2 polypeptides encoded by the nucleic acid. Consequently, the method covers the production of a very large variety of heterogeneous cell compositions that comprise protected neuronal cells.

Amount of direction or guidance presented and the presence or absence of working examples. The examples of the specification are limited to producing a cell composition from a mouse embryonal carcinoma cell line (P19 cells) transfected with an MEF2 nucleic acid molecule or mouse ES cell line (D3 cells) transfected with an MEF2 nucleic acid molecule. Cells expressing MEF2C exhibited a bipolar cell phenotype that expresses both neuronal (neurofilament) and myogenic (myosin heavy chain) markers (specification at page 68, paragraph 2). All experiments were in vitro assays. The specification does not provide examples of *in vivo* differentiation or *in vivo* transfection of progenitor cells. The specification teaches that the cell compositions produced from the claimed method can be used to treat a wide variety of neurodegenerative diseases, including Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Alzheimer's disease and other forms of dementia, multiple sclerosis,

epilepsy, and pain (pages 1-3). With regard to the use of the cell compositions produced, i.e. for treatment of a neurodegenerative disease, the specification provides only general guidance rather than specific guidance. With regard to *in vivo* uses of the method to produce a treatment effect, the specification provides little to no guidance. The specification does not assert a utility for the *in vivo* application of the method in the absence of a treatment effect. The specification does not offer specific guidance as to how the cell compositions produced can be used therapeutically for any given disorder. No working examples demonstrate a therapeutic effect upon transplantation of the claimed composition. Methods of treating neurodegenerative disorders by cell therapy or *in vivo* gene therapy are in their infancy. Therefore, considerable guidance is needed.

State of the prior art and predictability of the art. The specification fails to provide an enabling disclosure for the therapeutic use of the cell compositions produced from the claimed method. Thus, the specification fails to teach how to use the claimed invention for the only asserted utility. At the time the invention was made, successful implementation of cell therapy and gene therapy protocols was not routinely achievable by those skilled in the art.

Rossi and Cattaneo (2002) acknowledge that "despite intense research activities and media attention, stem cell therapy for neurological disorders is still a distant goal" (abstract). The reference emphasizes the need for homogeneous populations of neural stem cells and the further need to understand the mechanisms required for "their proper integration into the injured brain" (abstract). The authors point out that "the functional integration of donor cells remains a highly demanding task that requires a profound understanding and control of the biological properties of both donor cells and the host environment" (page 401, column 2, paragraph 2, last sentence).

Cao et al. (2002) acknowledge the potential for the use of stem cells in therapeutic transplantation and for *in vivo* manipulation of endogenous precursors, but emphasize that "this at present is challenging and so far has been unsuccessful" (abstract and page 507, column 2, paragraph 2). The authors further

point out that "[u]nderstanding mechanisms of NSC differentiation in the context of the injured CNS will be critical to achieving these therapeutic strategies" (abstract and page 507, column 2, paragraph 2). Given the unpredictability in the art of therapeutic transplantation, the development of therapeutic protocols requires substantial experimentation.

Mehler et al. (1999) disclose that many studies have suggested that the normal adult brain may lack the appropriate environmental signals to allow neural progenitors to realize their broad lineage potential. Specific neuropathologic conditions may alter the normal balance of regional environmental signals, for example by releasing proinflammatory and other modulatory cytokines. The presence of these inappropriate cellular cues may predispose residual neural populations to undergo apoptosis. The authors state that "[t]his suggests that it may be necessary to promote lineage commitment of progenitor cells *in vitro* prior to transplantation into a damaged brain" (p. 782, column 1, paragraph 1).

The specification fails to provide an enabling disclosure for the methods of making a cell population containing protected neuronal cells because the specification teaches that the only use for the compositions produced is for therapeutic transplantation, but methods of transplantation of neural tissue or other cells into the CNS or PNS are not routinely successful and the specification does not offer adequate guidance to enable one skilled in the art to practice the claimed invention to derive a therapeutic benefit in a diseased animal. The specification teaches that the only use for the compositions produced from the claimed method is for transplantation to produce a therapeutic effect but the specification does not adequately teach how to use the cell compositions produced by the claimed method to produce such an effect. Jackowski et al. (1995) details the limitations and unpredictability associated with the transplantation of neural tissue. At page 311, column 1, paragraph 2, the reference discusses the barriers to successful transplantation of neural tissue, notably the presence of molecules that actively inhibit the regeneration of mammalian CNS and PNS axons. Grados-Munro et al. (2003) further disclose that axon outgrowth inhibition is a major barrier to axon regeneration in the CNS. Various myelin-associated

inhibitors have been identified and their *in vivo* inhibitory effects have been characterized. The authors contemplate that a combination of approaches, including treatment to neutralize the inhibitory character of the CNS environment, may be required for CNS regenerative therapy (page 479). Other problems relating to appropriate environmental cues for axon guidance are also discussed. Filbin (2003) also discloses that inhibitors of axonal regeneration are present in the adult mammalian CNS and further discusses the inhibitory effect of glial scars which form after injury. Growth cone collapse is noted as the first event in inhibition of axonal growth and the response of neurons to inhibitors is discussed, including the current state of the art with regard to the intracellular inhibitory signalling pathway. Mehler et al. (1999) details the unpredictability and technical problems encountered in using progenitor cells for neural regeneration, particularly in the CNS. The authors state that "the reconstitution of more complex and widespread neural populations damaged by a variety of genetic or acquired neurological disorders such as stroke or traumatic injury will require access to a broader array of neural lineage species and a greater understanding of the developmental signals that sanction integration into the host environments. Many studies have suggested that the normal adult brain may lack the appropriate environmental signals to allow neural progenitor species present in multiple mature CNS regions to realize their broad lineage potential" (page 781, column 2, paragraph 2). The instant specification does not offer specific guidance as to how the full scope of the compositions produced by the claimed method could be used therapeutically for the treatment of any disorder, including Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), AIDS dementia complex, Rett Syndrome, epilepsy, ischemia, spinal cord damage, hepatic encephalopathy, Tourette's syndrome, or drug addiction, as contemplated in the specification. These diseases involve ongoing pathological processes that affect the survival or function of endogenous neurons as well as transplanted neurons. Neither the specification nor the prior art provides evidence that the cell compositions produced by the claimed method will provide a therapeutic effect in these environments upon transplantation.

Furthermore, neither the specification nor the prior art provides evidence that *in vivo* differentiation of progenitor cells residing *in vivo* can be used to provide a therapeutic effect in such disease environments. Moreover, Jackowski points out that membrane-associated or extracellular matrix-associated molecules that **inhibit** the successful regeneration of adult mammalian CNS and PNS axons are present in the CNS and PNS (page 311, column 1, paragraph 2).

The court has recognized that physiological activity is unpredictable. *In re Fisher*, 166 USPQ 18 (CCPA 1970). In cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with degree of unpredictability of factors involved. *In re Fisher*, 166 USPQ 18 (CCPA 1970).

In view of the quantity of experimentation necessary to determine appropriate parameters for using the resulting cell compositions to achieve a therapeutic outcome, and given the lack of applicable working examples directed to therapeutic transplantation, the limited guidance in the specification with regard to transplantation protocols and their applicability to pathologic conditions, the broad scope of the claims with regard to the wide variety of progenitor cells that may be used and the wide variety of cell compositions that may be developed from the claimed method, and further given the unpredictability in the art of therapeutic transplantation, undue experimentation would have been required for one skilled in the art to practice the claimed methods to make useful cell compositions and use the claimed method in vivo to achieve a therapeutic effect.

Given the lack of applicable working examples, the limited guidance provided in the specification, the broad scope of the claims with regard to the wide variety of progenitor cell types that could be used, the unpredictability for achieving a therapeutic effect upon the transplantation of the resulting cell compositions, and the unpredictability for carrying out the claimed method *in vivo*, undue experimentation would have been required for one skilled in the art to practice the claimed method of the invention in a human patient for therapeutic benefit.

Even as late as 2001, the art acknowledged that gene transfer into human hematopoietic stem cells was problematic (Hanazono et al., 2001). The claimed invention must be enabled at the time of filing. However, the priority date of this application is June 5, 2000. Thus, the instant specification must provide an enabling disclosure for the claimed invention as of this priority date.

The specification fails to provide an enabling disclosure for the genetic modification of human ES cells. The recent literature addresses the difficulties encountered in attempting to transfect human ES cells. Zwaka et al. (2003) points out that there are significant differences between mouse and human ES cells and that "[h]igh, stable transfection efficiencies in human ES cells have been difficult to achieve, and, in particular, electroporation protocols established for mouse ES cells work poorly in human ES cells" (abstract). Thus, it is clear that the behavior of mouse ES cells is not predictive of human ES cells. In April 2001, Eiges et al. compared the efficiency of several different transfection protocols for human ES cells. The reference demonstrates use of the transfection protocol of ExGen 500 to transfect human ES cells. However, the instant specification teaches the use of adenovirus transduction for the genetic modification of human ES cells. Example 6 of the specification describes the transfection of human ES cells with an adenovirus carrying the β-galactosidase reporter gene. Although the disclosure states that "[s]taining for expression of the β-galactosidase marker gene was performed," no results are provided with regard to the detection of β-galactosidase-expressing cells. Thus, at the time of filing, methods for successfully transfecting human ES cells were not known. The teachings of Eiges et al. (2001) would not have been available to the skilled artisan as of the filing date of this application which is June 5, 2000.

Regarding gene transfer into human HSCs, even as late as 2001, the art acknowledged that gene transfer into human hematopoietic stem cells was problematic (Hanazono et al., 2001). The claimed invention must be enabled at the time of filing. However, the priority date of this application is June 5,

2000. Thus, the instant specification must provide an enabling disclosure for the claimed invention as of this priority date.

The specification contemplates that transfecting the ES cells with a nucleic acid encoding an MEF2 and contacting the cells with a differentiating agent will be sufficient to direct the cells to differentiate *in vivo* or *in vitro* into the appropriate cell type and functionally integrate into the tissue into which they are implanted. However, the state of the art for *in vivo* differentiation of ES cells is undeveloped. While much work has been done to develop techniques for the directed differentiation of ES cells *in vitro* to produce desired cell types, little is known about the behavior of these cells *in vivo* or how they will interact with the local environment when implanted into adult tissues. Jackowski (1995) details the limitations and unpredictability associated with the transplantation of neural tissue.

Given the lack of applicable working examples, the limited guidance provided in the specification, the broad scope of the claims with regard to the wide variety of stem or progenitor cell types that could be used, and the unpredictability for producing cells suitable for therapeutic transplantation, undue experimentation would have been required for one skilled in the art to practice the claimed method of the invention to produce useful cell compositions.

At page 8 of the response, Applicants dismiss the teachings of Rossi and Cattaneo (2002) as not relevant to the claimed methods because Rossi and Cattaneo discuss neural stem cells. On the contrary, the reference aptly demonstrates the unpredictability in the field of cell therapy. As claimed, the method for producing protected neurons may be carried out *in vivo* or *in vitro* and the progenitor cells used as the starting material may be any type of progenitor cell, including neural stem cells. Thus, the reference is extremely relevant to the claimed invention. The claims recite the use of "progenitor cells" and "human stem cells." Both terms include neural stem cells. The specification does not provide a special definition for the term "progenitor cells," such that the terminology excludes neural stem cells. It is clear that the

term "progenitor cells" includes all types of stem cells, including neural stem cells, hematopoietic stem cells, embryonic stem cells, embryonic carcinoma cells, and others.

At pages 9-10 of the response, Applicants dismiss the teachings of Cao et al. (2002) as not relevant to the claimed methods reciting specific steps for differentiating cells *in vitro* or *in vivo*. Applicants assert that "Cao et al. discusses the use of neural stem cells for repair of central nervous system injury, not the use of stem cells in general." However, Cao et al. need not discuss stem cells in general to demonstrate the unpredictability in the art, particularly in methods where progenitor cells are to be used directly or indirectly for purposes of therapeutic transplantation. As Applicants acknowledge, the instant specification teaches that cells can be transplanted into a patient prior to, during or after differentiation of the progenitor cells into neuronal cells and that the neuronal environment can drive the cells into the desired cell type due to the presence of the appropriate environmental cues (page 58, line 29 to page 59, line 11). Applicants assert that Cao et al. describes the *in vivo* manipulation of endogenous precursors. Applicants point specifically to two passages, as follows:

> Manipulation of endogenous neural precursors may be an alternative therapy or a complimentary therapy to stem cell transplantation for neurodegenerative disease and CNS injury. However, this at present is challenging and so far has been unsuccessful. Understanding mechanisms of NSC differentiation in the context of the injured CNS will be critical to achieving these therapeutic strategies. (Abstract)
>
> There is great potential for the ultimate manipulation and directed differentiation of endogenous stem cell populations, but this is at present a challenging and as yet unsuccessful approach (page 507, column 2, paragaph 2).

Applicants assert that "Cao et al. is referring to mechanisms of neural stem cell differentiation of endogenous neural stem cells" (emphasis original) and that "[i]n contrast, the claimed methods are directed to differentiating progenitor cells by contacting the progenitor cells with a differentiating agent and introducing a nucleic acid encoding a MEF2 polypeptide." However, the presently claimed invention also is directed, in relevant part, to the *in vivo* manipulation of endogenous precursors. As noted in the rejection of record, the progenitor cell may be in culture or may be an endogenous cell residing *in vivo*

(page 3, lines 1-2 of the Office Action mailed 8/12/05). Thus, the claims clearly cover contacting an endogenous neural stem cell, or any other endogenous progenitor cell, with a differentiating agent and introducing a nucleic acid encoding a MEF2 polypeptide into the endogenous neural stem cell, or other endogenous progenitor cell. The specification provides the explicit teaching that

> "a progenitor population in which the p38 MAP kinase/MEF2 pathway has been induced can be transplanted into a patient prior to, during or after differentiation of the progenitor cells into neuronal cells. In one embodiment, cells are transplanted prior to or during differentiation. Where cells are transplanted prior to differentiation, the neuronal environment can drive the cells into the desired neuronal cell type, rather than, for example, muscle cells due to the presence of the appropriate environmental cues. In view of the above, it is clear that differentiation can occur *in vitro* or *in vivo*, or can occur partially *in vitro* and partially *in vivo*." (page 58, line 29 to page 59, line 11)

As noted in the rejection of record, the claims encompass *in vivo* and *in vitro* applications of the method. The method steps can be carried out entirely *in vivo*, entirely *in vitro*, or partially *in vitro* and partially *in vivo*. The contacting step and introducing step may be carried out in any order. Thus, the teachings of Cao et al. are on point and extremely relevant to the instantly claimed invention. The reference demonstrates the unpredictability of both therapeutic transplantation and the *in vivo* manipulation of endogenous precursors for therapeutic purposes.

At page 10 of the response, with regard to Milward et al. (1997), Applicants argue that the reference is completely silent as to any therapeutic effect of the transplanted cells in either the rat or dog model and makes no statement that production of myelin *in vivo* is not predictive of a therapeutic outcome. While it is acknowledged that the reference does not explicitly state that there was no therapeutic effect, nevertheless, the reference does not report that a therapeutic effect was achieved.

At page 11 of the response, with regard to Mehler et al. (1999), Applicants dismiss the teachings of the reference as not relevant to the claimed invention. Applicants refer to the teachings that show that many studies have suggested that the normal adult brain may lack the appropriate environmental signals to allow neural progenitors to realize their broad lineage potential and that specific neuropathological conditions may alter the normal balance of regional environmental signals and that inappropriate cellular

cues may predispose residual neural populations to undergo apoptosis. In response, Applicants assert that the claimed methods are directed to differentiating progenitor cells by contacting the progenitor cells with a differentiating agent. Applicants conclude that the issues with respect to environmental cues for differentiation of progenitor cells are not relevant to the claimed methods reciting specific steps for differentiating cells *in vitro* or *in vivo*. On the contrary, with the exception of Claims 18, 19, 76, and 77, the claims do not recite specific steps for differentiating cells as Applicants now contend, but instead recite "contacting said progenitor cells with a differentiating agent." When the claims are given their broadest reasonable interpretation, the differentiating agent can be the *in vivo* environment of the brain, for example. The specification explicitly states that

> "Where cells are transplanted prior to differentiation, the neuronal environment can drive the cells into the desired neuronal cell type, rather than, for example, muscle cells due to the presence of the appropriate environmental cues. In view of the above, it is clear that differentiation can occur *in vitro* or *in vivo*, or can occur partially *in vitro* and partially *in vivo*." (page 59, lines 4-11)

Thus, it cannot be said that the issues with respect to environmental cues for differentiation of progenitor cells are not relevant to the claimed methods.

At page 12 of the response, Applicants assert that Cheng et al. (1998) described "an optimized gene-transfer protocol clinically applicable to gene transfer into human hematopoietic cells" (emphasis original). Applicants go on to assert that the gene transfer protocol described by Cheng et al. is relevant to *in vivo* uses since such viral vectors are routinely used for *in vivo* transduction. Applicants assert that Hanazono et al. acknowledges that such vectors are routinely used for *in vivo* transduction. This is not accurate. Hanazono et al. states that "[t]hese vectors are being examined *in vivo* in nonhuman primate models" (page 16, column 2, paragraph 1). The Examiner does not find that Hanazono et al. acknowledge that such vectors are routinely used for *in vivo* transduction. Protocols for using MSCV to transfect human hematopoietic stem cells *in vivo* was not taught by Cheng et al. The instant claims encompass *in vivo* transduction of human hematopoietic stem cells, in the context of therapeutic

protocols. As late as 2001, the art acknowledged that gene transfer into human hematopoietic stem cells was problematic (Hanazono et al., 2001). The claimed invention must be enabled at the time of filing. However, the priority date of this application is June 5, 2000. Thus, the instant specification must provide an enabling disclosure for the claimed invention as of this priority date.

At pages 11-12 of the response, Applicants assert that the specification provides sufficient description and guidance to enable the genetic modification of human ES cells. Citing pages 54-55, Applicants assert that the specification teaches that well known methods for introducing a nucleic acid molecule into progenitor cells such as embryonic stem cells include microinjection, electroporation, and lipofection as well as viral-mediated techniques such as retroviral, lentiviral and adenoviral transduction. However, the guidance provided in the specification is in the form of general guidance rather than specific guidance. When methods for the genetic modification of human embryonic stem cells are not known in the prior art, specific guidance is needed to enable the invention. The only specific guidance with regard to the genetic modification of human ES cells in particular is provided in the examples. Example 6 teaches using adenovirus transduction for the genetic modification of human ES cells, but no results are provided for the adenovirus transduction experiments.

At page 13 of the response, Applicants assert that Zwaka and Thomson (2003), a post-filing reference, corroborates their position that electroporation can be used to produce stably transfected human ES cells. Applicants acknowledge that Zwaka and Thomson is a post-filing reference that describes an improved electroporation method that was specially adapted for human ES cells. Nevertheless, Applicants assert that Zwaka and Thomson teach the use of known electroporation techniques that were successful for transfecting human ES cells, albeit at lower efficiencies than their improved technique. However, the instant specification does not provide specific guidance for using electroporation techniques for transfecting human ES cells. With regard to embryonic stem cells in general, the specification provides only a laundry list of techniques that could be used to introduce a nucleic acid molecule into an

embryonic stem cell. With regard to human ES cells specifically, the only specific guidance for their genetic modification is provided in the examples. Example 6 teaches using adenovirus transduction for the genetic modification of human ES cells, but no results are provided for the adenovirus transduction experiments.

At pages 14-15 of the response, Applicants assert that Eiges et al. (2001) showed that protocols using the transfection reagents Fugene and ExGen 500 were successful for the genetic modification of human ES cells, with ExGen 500 showing the highest efficiency. Applicants further assert that Ferrari et al. (1997) and Uyttersprot et al. (1998) demonstrate that transfection methods using Fugene and ExGen 500 were available to those skilled in the art at the time of filing the priority application on June 5, 2000. Applicants submit that it would have been routine for one skilled in the art to try various known transfection methods to successfully transfect human ES cells. However, when take as a whole, in combination with the limited guidance of the specification and other unpredictabilities in the art, the lack of a known and available transfection method for human ES cells is clearly an additional obstacle that must be overcome to enable the claimed invention which is very broad in terms of the types of progenitor cells and stem cells that can be used in carrying out the claimed method. The instant specification provides no specific guidance with regard to the genetic modification of human ES cells and does not mention either FUGENE or ExGen 500 transfection reagents as being particularly suitable for the genetic modification of human ES cells. Ferrari et al. (1997) and Uyttersprot et al. (1998) likewise do not suggest using FUGENE or ExGen 500 for the genetic modification of human ES cells. Ferrari et al. teaches the transfection of lung epithelial cells by a chemical transfection method that uses ExGen 500. Uyttersprot et al. teaches the transfection of dog and human thyrocytes by a chemical transfection method that uses the transfection reagent FUGENE. Thus, at time of filing the priority application, neither the specification nor the prior art disclosed a method for the genetic modification of human ES cells. The only specific guidance offered by the specification for the genetic modification of human ES cells was

that of Example 6 which teaches using adenovirus transduction for the genetic modification of human ES cells. In the example, human ES cells were transduced with an adenovirus carrying the β-galactosidase reporter gene. Although the disclosure states that "[s]taining for expression of the β-galactosidase marker gene was performed," no results are provided with regard to the detection of β-galactosidase-expressing cells. Thus, at the time of filing, methods for successfully transfecting human ES cells were not known. This is a factor that must be taken into consideration when evaluating enablement across the full scope of the claims. When considered in combination with the other factors discussed in the rejection of record, the conclusion is that undue experimentation would have been required to practice the claimed invention across the full scope.

At page 15 of the response, Applicants assert that antibiotic selection can be used *in vitro* to select transfected progenitor cells and therefore a high efficiency of transfection of human or other progenitor cells is not needed. Applicants maintain that cell populations stably expressing an introduced nucleic acid can be routinely prepared using standard method such as antibiotic selection in order to select for a transfected population of cells. Applicants conclude that the skilled person understands that, even if progenitor cells were not transfected with particularly high efficiency, one skilled in the art would have been able to produce a population of progenitor cells predominantly or uniformly containing a MEF2 polypeptide using only routine methods. With the exception of human ES cells and human hematopoietic stem cells, the Examiner acknowledges that, when the claimed method is being carried out *in vitro*, one skilled in the art could use antibiotic selection techniques to obtain a population of progenitor cells expressing MEF2. Nevertheless, when transfection efficiencies are low, the number of cells obtained upon selection with antibiotic will also be low. Given that the specification makes it clear that the protected neuronal cells are being produced for use in transplantation protocols to treat a variety of diseases, it is not evident that low cell numbers would be sufficient to treat even a single patient. The primary problem in many transplantation protocols is to obtain a sample containing a sufficient number of

neurons to treat a patient. It is therefore not evident, as Applicants continue to argue, that low transfection efficiencies would be sufficient, even with antibiotic selection to remove non-transfected cells, to produce cell populations suitable for therapeutic transplantation, the only disclosed utility for the protected neuronal cells. Obviously the same problem pertains to *in vivo* transfection protocols, which the claims also cover. If the transfection protocol is not capable of transfecting a sufficient number of cells to produce a therapeutic effect, then low transfection efficiencies would not be considered enough to enable the claimed invention over the full scope. In the instant case, neither the prior art nor the instant specification teaches the number of cells that would be needed to produce a therapeutic effect for any of the diseases mentioned in the specification. This is an additional unpredictability that, in combination with the other factors discussed in the rejection of record, leads to a conclusion that undue experimentation would be required to practice the claimed invention over the full scope.

At pages 15-16 of the response, Applicants assert that differentiation can occur *in vivo* in injury and disease. As an example, Applicants point to Milward et al. for describing differentiation of transplanted neurospheres into oligodendrocytes in a myelin-deficient rat model, which Applicants contend is an ongoing pathological process, not an injury model. Contrary to this contention, the myelin deficiency is not the result of an ongoing pathological process, but is the result of a genetic defect which prevents the formation of myelin during development. There is no ongoing pathological process that attacks the myelin as it is formed. Furthermore, this model system only demonstrates the differentiation of neural stem cells to **oligodendrocytes** in the *in vivo* environment. It does not demonstrate the differentiation of progenitor cells to **neurons** in the *in vivo* environment, as instantly claimed. Nevertheless, there is no suggestion that differentiation of progenitor cells to neurons can never occur in the *in vivo* disease environment of any and all neurodegenerative diseases. The issue is whether the instant specification, in combination with the prior art, provides sufficient specific guidance to enable one of skill in the art to carry out the claimed method in all disease environments, using any type of progenitor

cell and any type of differentiating agent. Here, it does not. The claims cover *in vivo* differentiation of any and all types of progenitor cells and stem cells in any and all *in vivo* disease environments. The art of record demonstrates the unpredictability and the inhibitory processes that prevent axonal growth cones from extending to appropriate partners in both the *in vivo* disease environment and healthy environment. Cell survival is also an issue in the disease environment, particularly in those where an ongoing pathological process is continuing to attack neurons and differentiating progenitor cells in the local region.

At page 16 of the response, Applicants submit that Rossi and Cattaneo (2002) corroborate Applicants' position that differentiation can occur in disease and injury. Applicants point to the discussion pertaining to Huntington's disease and the transplantation of fetal cells. While limited successes have been obtained using cells isolated from fetal and embryonic sources, these limited successes were achieved through painstaking and intensive research effort, not routine experimentation. The evidence of record does not demonstrate that these results could be extended or extrapolated to the claimed invention, to enable the full scope, using nothing more than routine experimentation. The claimed invention is not directed to preparing primary cell explants from fetal tissue. The claimed invention is direct to preparing neuronal cell compositions from a wide variety of "progenitor" cells or *in vivo* differentiation of "progenitor" cells in a therapeutic context. The cell compositions developed from these progenitor cells are quite distinct from fetal primary cell explants. As Jackowski points out fetal neurons possess certain properties that other cells do not. The prior art references in combination with the instant specification do not teach which progenitor cells will differentiate and integrate in which disease environments. Given the rejection of record, it cannot be said that all disease environments are permissive for *in vivo* differentiation of any and all types of progenitor cells, as covered by the claims. The teachings of Rossi and Cattaneo are specific to Huntington's disease and fetal grafts and there is no

evidence of record that nothing more than routine experimentation would be required to extend the result to all the diseases mentioned in the specification and all the progenitor cell types covered by the claims.

At page 17 of the response, Applicants refer to the Declaration of Dr. Stuart Lipton, filed on February 23, 2005. The declaration has been fully considered but is not found to be persuasive for the following reasons. The Declaration does not provide a nexus between the experiments described therein and the teachings of the specification. The Declaration refers to mice injected with MEF2CA-ES-derived neural progenitors labeled with BrdU. There is no information in either the Declaration or the instant specification describing how these ES-derived neural progenitors were obtained or produced. It is unclear if any differentiating agent was used to "derive" the neural progenitors from the ES cells. The term MEF2CA is not used in the instant specification and therefore it is impossible to identify a nexus between the Declaration and the teachings of the specification. As a further issue, the Declaration states that the injected cells were labeled with BrdU, but the remainder of the Declaration goes on to describe brain sections stained with anti-GFP antibodies to identify the transplanted cells. It is unclear where the GFP-expressing cells are coming from. The Declaration states that "[a]t day 1 post-transplantation, **all** of the grafted cells expressed EGFP." However, there is no indication that cells comprising an EGFP vector were transplanted into the mice. Thus, it is unclear where the EGFP is coming from and why it is down regulated in the days following engrafting. As further issue, the Declaration refers to Exhibit 3, Figure 5-3 with various colors indicating different molecular stains. However, color photographs were not provided and none of the features described are visible. For the above reasons, it is impossible to determine if the experiments described in the Declaration were carried out in accordance with the teachings of the specification. Thus, there is no nexus between the Declaration and the instant specification and therefore the Declaration is not found to be persuasive.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-20 and 58-79 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-20 and 58-79 are indefinite in their recitation of "protected neuronal cells." The metes and bounds of the term "protected" in this context are not clearly set forth. At pages pages 16-17, the specification states "[t]he present invention relates to the finding that the p38α/MEF2 pathway plays an important role in preventing apoptotic cell death during neuronal differentiation. Based on this finding, the present invention provides a method of differentiating progenitor cells to produce a population of neuronal cells which is protected from apoptotic cell death." While it is clear that the term protected includes cells that are protected from apoptotic cell death, the term "protected" is much broader and the metes and bounds are not clearly set forth. Thus, it is unclear what other characteristics would be sufficient to constitute a "protected neuronal cell." The specification does not provide a definition for the term.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-4, 18, 58-62, 76, and 79 are rejected under 35 U.S.C. 102(a) as being anticipated by Okamoto et al. (2000, Proc. Natl. Acad. Sci. 97(13): 7561-7566, published online June 13, 2000).

The claims are directed to a method of differentiating progenitor cells by (a) contacting said progenitor cells with a differentiating agent, and (b) introducing into said progenitor cells a nucleic acid molecule encoding a MEF2 polypeptide or an active fragment thereof, thereby differentiating said progenitor cells to produce a cell population containing protected neuronal cells.

Okamoto et al. (2000) discloses P19 cells treated with retinoic acid and subsequently transfected with a plasmid encoding a constitutively active form of MEF2C (page 7563, column 1, paragraph 2). The reference further discloses that transfection of P19 cells with an expression vector encoding a dominant negative form of p38α resulted in enhanced apoptotic cell death in differentiating cells (page 7565, column 2, paragraph 2). Coexpression of constitutively active MEF2C rescued the differentiating cells from apoptosis (Figure 5B and page 7565, column 2, paragraph 2). The authors conclude that the p38α/MEF2 cascade plays a role in preventing apoptotic cell death during neuronal differentiation. The neurons expressing constitutively active MEF2 represent a population of protected neuronal cells, as presently claimed.

Thus, the claimed invention is disclosed in the prior art.

Claims 1, 2, 18, 58-60, 76, and 79 are rejected under 35 U.S.C. 102(b) as being anticipated by Krainc et al. (1998, J. Biol. Chem. 273(40): 26218-26224).

The claims are directed to a method of differentiating progenitor cells by (a) contacting said progenitor cells with a differentiating agent, and (b) introducing into said progenitor cells a nucleic acid molecule encoding a MEF2 polypeptide or an active fragment thereof, thereby differentiating said progenitor cells to produce a cell population containing protected neuronal cells.

Krainc et al. (1998) disclose that the plasmid pG/DN, containing theN-terminal DNA binding domain of MEF2C, was stably transfected into P19 cells (Figure 5 and page 26222, column 2, paragraph

2). The reference notes that these cells differentiate into a neuronal phenotype after treatment with retinoic acid, and then express MEF2C (page 26222, column 2, paragraph 2).

Thus, the claimed invention is disclosed in the prior art.

Claims 1, 18, 20, 59, 76, and 78 are rejected under 35 U.S.C. 102(b) as being anticipated by Mao et al. (1996, J. Biol. Chem. 271(24): 14371-14375).

The claims are directed to a method of differentiating progenitor cells by (a) contacting said progenitor cells with a differentiating agent, and (b) introducing into said progenitor cells a nucleic acid molecule encoding a MEF2 polypeptide or an active fragment thereof, thereby differentiating said progenitor cells to produce a cell population containing protected neuronal cells.

Mao et al. (1996) disclose the transfection of the mouse teratocarcinoma cell line P19 with a plasmid encoding MEF2A. P19 cells were induced to differentiate along the neuronal lineage by exposure to retinoic acid. The expression of MEF2A and MASH1 during retinoic acid-induced P19 neuronal differentiation is reported in Table 1 (page 14373). The reference further discloses that MEF2A and MASH1 are coordinately induced during the differentiation of P19 cells along a neuronal lineage and that in transient transfection assays, MEF2A and MASH1 cooperatively activate gene expression (abstract).

Thus, the claimed invention is disclosed in the prior art.

Claims 1, 2, 18, 58-60, 76, and 79 are rejected under 35 U.S.C. 102(b) as being anticipated by Skerjanc et al. (4/21/2000, FEBS Letters 472(1): 53-63), as evidenced by Skerjanc et al. (1998, J. Biol. Chem. 273(52):34904-34910).

The claims are directed to a method of differentiating progenitor cells by (a) contacting said progenitor cells with a differentiating agent, and (b) introducing into said progenitor cells a nucleic acid

molecule encoding a MEF2 polypeptide or an active fragment thereof, thereby differentiating said progenitor cells to produce a cell population containing protected neuronal cells.

Skerjanc et al. (2000) disclose that mouse P19 cell lines overexpressing MEF2C differentiated into neural cells in the presence of DMSO (abstract; page 54, column 2, paragraph 2; and Figure 2). At page 53, column 2, paragraph 3, the reference discloses that P19 cells overexpressing MEF2C, termed P19[MEF2C] cells, were described previously by Skerjanc et al. (1998). The 1998 reference discloses that stable cell lines expressing MEF2C were isolated upon transfection of P19 cells with 6.5 μg of a plasmid containing the *pgk-1* promoter and the coding sequence of human MEF2C, designated PGK-MEF2C (page 34905, column 1, paragraph 5). Thus, the P19 cell line was stably transfected with the MEF2C gene. The 2000 reference discloses that MEF2C can induce neurogenesis when overexpressed in P19 cells (page 53, column 1, paragraph 2 and abstract) and that neurons were observed in P19[MEF2C] cultures when aggregated with DMSO and that the neurons appeared to have a similar morphology when compared to neurons induced by retinoic acid (page 54, column 2, paragraph 1).

Thus, the claimed invention is disclosed in the prior art.

Conclusion

No claims are allowable.

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Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anne-Marie Falk whose telephone number is (571) 272-0728. The examiner can normally be reached Monday through Friday from 9:00 AM to 5:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras, can be reached on (571) 272-4517. The central official fax phone number for the organization where this application or proceeding is assigned is (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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PRIMARY EXAMINER